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Circulating level of α_2-macroglobulin–β_2-microglobulin complex in hemodialysis patients

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Circulating level of α_2-macroglobulin–β_2-microglobulin complex in hemodialysis patients.

Background. The presence of α_2-macroglobulin (α_2M) in amyloid tissue from patients with dialysis-related amyloidosis (DRA) was demonstrated by Argilés et al in 1989. Thereafter, the formation of the complex of β_2-microglobulin (β_2m) with α_2m was confirmed directly by in vitro study. In Alzheimer's disease, complex formation of amyloid β-peptide and α_2M is considered to play an important role in the pathogenesis by modifying the degradation processes of amyloid protein. Thus, we hypothesized that the α_2M-β_2m complex is an important factor in the pathogenesis of DRA as well. Here, we measured the circulating levels of α_2M-β_2m complex in the maintenance hemodialysis patients and discussed about its clinical significance in DRA.

Methods. One hundred and thirty-seven hemodialysis patients and 11 prehemodialysis chronic renal failure (CRF) patients were included in this study. The affinity of purified α_2M for β_2m was confirmed by a highly sensitive 27 MHz quartz crystal microbalance (QCM). The presence of circulating α_2M-β_2m complex was analyzed by immunoblotting analysis. Furthermore, the serum levels of α_2M-β_2m complex were measured by sandwich enzyme immunoassay.

Results. QCM analysis revealed the high affinity of α_2M for β_2m. The presence of circulating α_2M-β_2m complex was detected in two out of a total 11 prehemodialysis CRF patients and in 95 out of the total of 137 hemodialysis patients. None of the healthy subjects, however, were observed to present with any α_2M-β_2m complex. Serum levels of the α_2M-β_2m complex were correlated to the duration of hemodialysis ($P = 0.043$). Serum levels of the α_2M-β_2m complex were significantly higher in patients with high DRA score than in patients with negative DRA score ($P = 0.018$). Moreover, serum levels of the α_2M-β_2m complex showed significantly lower in the hemodiafiltration patients compared to the hemodialysis patients ($P = 0.002$) and showed a strong correlation with DRA score in hemodialysis patients excluding 11 hemodiafiltration patients ($P = 0.0004$).

Conclusion. This study is the first to demonstrate the presence of circulating α_2M-β_2m complex in hemodialysis patients. Furthermore, we observed the correlation between serum levels of α_2M-β_2m complex and clinical characteristics of DRA. Thus we concluded that a formation of an α_2M-β_2m complex may be implicated in DRA.

Key words: hemodialysis, α_2-macroglobulin-β_2-microglobulin complex, dialysis-related amyloidosis.

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α_2-macroglobulin (α_2M) is a major protease inhibitor in vivo [1, 2]. So far, several reports have been published suggesting the presence of protease inhibitors such as α_2M and α_1 antichymotripsin in amyloid fibrils from Alzheimer's disease or dialysis-related amyloidosis (DRA) patients [3–6]. More recently, the very potent inhibitory action of α_2M on proteases has been interpreted as being indicative of an involvement of α_2M in amyloid-related protein metabolism. In consequence of this, it has been hypothesized that α_2M may modify the degradation processes of amyloid proteins and may play an active role in the pathogenesis of amyloidosis.

In Alzheimer's disease, amyloid β peptide, a precursor protein of the disease, has now been corroborated to form a complex with α_2M in in vitro studies [7, 8].

Subsequently, β_2-microglobulin (β_2m), a precursor protein of DRA, has been reported to form a complex with α_2M [9]. On the basis of this evidence, the "α_2M hypothesis" has been forwarded by Argilés et al [10] as an interesting mechanism to account for the pathogenesis of DRA. Based on this complex-mediated promotion concept, we established an assay system using the enzyme immunoassay method for α_2M-β_2m complex and measured its serum level in 137 hemodialysis patients as well as 11 prehemodialysis patients.

METHODS

Subjects

One hundred and thirty-seven hemodialysis patients, 11 prehemodialysis patients with chronic renal failure

(CRF) (control subjects, prehemodialysis CRF patients), and 15 normal persons were enrolled in this study after obtaining their oral consent.

All patients were nondiabetic. Their average ages were 58 years in the hemodialysis patients, 61 years in the control subjects, and 33 years in the normal persons. Gender breakdown was 77 males and 60 females of the hemodialysis patients, nine males and two females of the prehemodialysis CRF patients, and 12 males and three females of normal persons. Duration of hemodialysis varied from 2 to 290 months, 123 months in average. All hemodialysis patients underwent hemodialysis or hemodiafiltration with bicarbonate dialysate twice or three times a week. Several kinds of dialyzers were used at the time of the study, including polysulfone ($N = 45$), polyacrylonitrile ($N = 36$), ethylenevinylalcohol ($N = 31$), cellulose triacetate ($N = 20$), and others ($N = 5$).

The clinical diagnosis of DRA was evaluated as previously reported [11]. In brief, all clinical signs were scored by the criteria of Gejyo et al [12], which ranks the clinical features of DRA from 0 to 7 according to joint pain, bone cyst on x-ray film, and carpal tunnel syndrome. The DRA scores were negative in 84 cases (0 in 58 cases, 1 in 26 cases), mild in 33 cases (2 in 21 cases, 3 in 12 cases), and high in 20 cases (4 in ten cases, 5 in five cases, 6 in three cases, and 7 in two cases).

The operation for carpal tunnel syndrome had been done in 20 patients, the amyloid bone cyst in either carpal, shoulder, or hip bone was confirmed in 70 patients and 47 patients showed symptoms of multiple joint pain. Eleven patients who had a moderate or high DRA score had been undergoing hemodiafiltration for 6 months or more.

As far as we could survey, there were no reports concerning the complex of α_2M-β_2m even in the patients with the systemic AA amyloidosis. Therefore, age-matched prehemodialysis CRF patients served as control in this study.

Laboratory characteristics

Serum creatinine values were 12.0 ± 0.3 mg/dL in the hemodialysis patients and 5.4 ± 2.3 mg/dL in the prehemodialysis CRF patients. Serum values of β_2m were 35.7 ± 8.5 mg/L in the hemodialysis patients and 10.7 ± 6.0 mg/L in the prehemodialysis CRF patients. Values of serum total protein, albumin, C-reactive protein, and concentration of hemoglobin were 6.5 ± 0.4 g/dL, 3.8 ± 0.3 g/dL, 0.24 ± 0.31 mg/dL, and 10.0 ± 1.2 g/dL, respectively, in hemodialysis patients. All data in hemodialysis patients were values before hemodialysis.

Measurement of a circulating α_2M-β_2m complex

The affinity of β_2m for α_2M. The affinity of β_2m for α_2M was examined by using a highly sensitive 27 MHz quartz crystal microbalance (QCM) (Affinix Q) (Inishiamu Co., Tokyo, Japan) as described earlier [13, 14]. Briefly, 2 μL of 200 μg/mL α_2M was directly immobilized on a QCM plate, and the plate was soaked in 6 mL of 10% dimethyl sulfoxide (DMSO) at 25°C. The resonance frequency of the QCM was defined as the 0 position after equilibrium. The stability and drift of the 27 MHz QCM frequency in the solution were ± 5 Hz for 12 hours at 25°C.

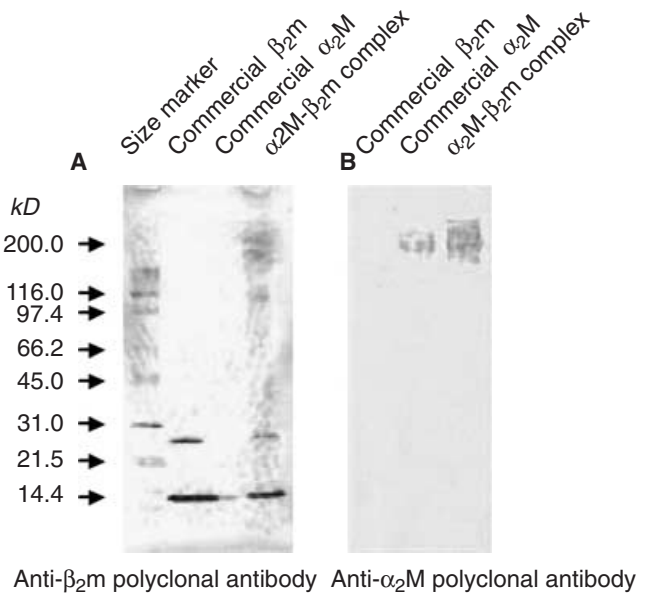

Fig. 1. Western blotting of the α_2-macroglobulin (α_2M)-β_2-microglobulin (β_2m) complex in vitro. Commercial α_2M, commercial β_2m and α_2M-β_2m complex were submitted to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and blotted with anti-β_2m polyclonal antibody (*A*) or anti-α_2M polyclonal antibody (*B*).

Preparation of standard α_2M-β_2m complex. A α_2M-β_2m complex was prepared as described previously [9]. Other chemicals were of analytic grade. In outline, 1 mL of 0.1 mg/mL β_2m (Sigma Chemical Co., St. Louis, MO, USA) was incubated with 1 mL of 1 mg/mL α_2M (Sigma Chemical Co.) for 36 hours in 66.6 mmol/L phosphate buffer at room temperature. Specificity of the standard complex was studied by Western blotting as shown in Figure 1. The complex was used as a sample for Western blotting or a standard for enzyme immunoassay.

Western blotting. Prepared α_2M-β_2m complex, commercial β_2m, and commercial α_2M were run by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) at 18 mA for $1^1/_2$ hours with a 4% to 20% gradient gel (Tefco, Tokyo, Japan). Prepared α_2M-β_2m complex was pretreated at 100°C for 5 minutes by mixing with 2-mercaptethanol of 5% final concentration. After transfer to a polyvinylidene difluoride (PVDF) membrane (Millipore, Bedford, MA, USA) for 1 hour at 20 V, the membrane was incubated with Block Ace (Dainippon Pharmaceutical, Osaka, Japan) for 1 hour

followed by three washes with 20 mmol/L phosphate-buffered saline (PBS) (pH 7.4) containing 0.05% Tween 20 (washing buffer). The membrane was then incubated with biotin-labeled anti-α_2M antibody or anti-β_2m antibody at room temperature for 1 hour. After the reaction, the membrane was washed three times with wash buffer and soaked by shaking in 5 mL of PBS containing an avidin-peroxidase-labeled biotin complex (Vectastain ABC Kit) (Vector, Burlingame, CA, USA) at room temperature for 30 minutes. Thereafter, the membrane was washed three times repeatedly with the wash buffer and reacted with nitrotetrazolium blue solution containing 0.02% hydrogen peroxide and the reduced form of nicotinamide adenine dinucleotide (NADH).

Biotin labeling of anti-α_2M antibody. Biotinylation of anti-α_2M polyclonal antibody (Rockland) (Gilbertsvilles, PA, USA) was performed using sulfosuccinimidyl D-biotin (Biotin Sulfo-OSu) (Dojindo, Kumamoto, Japan). First, 1 mL of the antibody solution (0.7 mg/mL) in 0.01 mol/L hepes buffer (pH 8.5) was prepared. Next, 0.1 mL of 1 mmol/L Biotin Sulfo-OSu in distilled water was prepared and then added to the antibody solution. After complete mixing, the solution was incubated at room temperature for 4 hours. The biotinylated antibody solution was loaded into the desalting column (R1).

A coupling anti-β_2m antibody to horseradish peroxidase. Five milligrams of horseradish peroxidase (HRP) (Toyobo, Osaka, Japan) was suspended in 1.2 mL of water and added to 0.3 mL of a freshly prepared 0.1 mol/L sodium periodate solution in 10 mmol/L sodium phosphate (pH 7.0). After the HRP solution had been incubated at room temperature for 20 minutes, it was dialyzed versus 1 mmol/L sodium acetate (pH 4.0) at 4°C with several changes overnight. The HRP solution was removed from the dialysis tube, added to 0.5 mL of anti-β_2m antibody solution, then prepared to 10 mg/mL in 20 mmol/L carbonate buffer (pH 9.0). Next, the HRP antibody solution was incubated at room temperature for 2 hours. After adding 0.1 mL of sodium borohydride (4 mg/mL in water) (Wako, Osaka, Japan) to the HRP antibody, the solution was incubated at 4°C for 2 hours. The HRP antibody solution was then dialyzed by several changes of 10 mmol/L sodium PBS (pH 7.4).

Two-step sandwich enzyme immunoassay. Measurement of the α_2M-β_2m complex in serum was performed by two-step sandwich enzyme immunoassay (two-step EIA) using full automatic analysis equipment MI01 (A & T, Kanagawa, Japan). A principle of assay followed two steps: (*1*) α_2M in serum was captured on reaction cup by biotin labeled anti-α_2M antibody through antibiotin antibody, (*2*) β_2m of the complex was detected by HRP-labeled anti-β_2m antibody. In the first step, the reaction cup used for immobilizing antibiotin polyclonal antibody was coated with antibiotin polyclonal antibody (Nippon Chemiphar, Tokyo, Japan) at 2.5 μg/cup prior to measurement. Twenty microliters of biotin-labeled anti-α_2M antibody solution (R1), containing 1% bovine serum albumin (BSA), 1% mouse serum, and 0.03% Microcide I (Amresco, Solon, OH, USA) in 20 mmol/L PBS, and 50 μL of the sample were mixed and preincubated for 5 minutes at 45°C in tube. Then, 50 μL of the R1 sample mixture was transferred to the reaction cup. Twenty microliters of HRP-coupled anti-β_2m antibody (R2), containing 1% BSA, 1% mouse serum, and 0.03% Microcide I (Amresco) in 20 mmol/L PBS, was added to the reaction cup (second step). The reaction cup contents were then incubated for 2 minutes at 45°C and washed two times with 0.05% Tween 20 solution. After the addition of 30 μL of chromogen (A & T) which consists of 3,3′,5,5′-tetramethylbenzidine and hydrogen peroxide, changes of laser reflectance were measured.

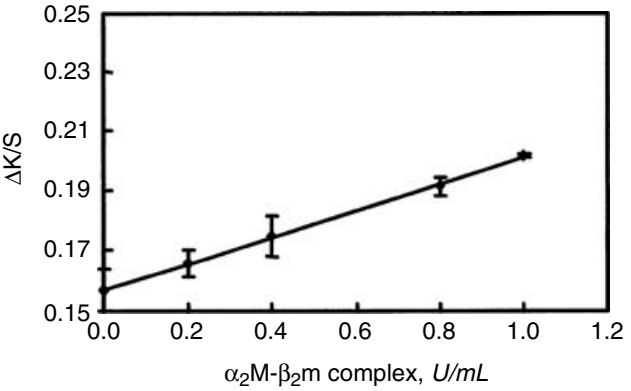

Fig. 2. A reference line of two-step enzyme imuunoassay. Abbreviations are: α_2M-β_2m complex, α_2-macroglobulin-β_2-microglobulin complex; ΔK/S, change for 1 minute of a ratio of the Kubelka-Munk coefficients; K is absorption and S is scattering.

The α_2M-β_2m complex, prepared in vitro, was used as the standard for the changes of laser reflectance. The sera of normal persons, hemodialysis, and prehemodialysis CRF patients were used as samples. The complex concentration of the samples was calculated with an MI01 (A & T) by fitting the standard curve using a spline function. The reference line showed a good linearity from 0 to 1.0 U/mL (Fig. 2). The intra-assay coefficient of variation was 4.67%.

Statistical analysis

Results are expressed as mean ± SD. Statistical analysis was performed using the software package, Stat View version 5.0 for Windows. Differences between the groups were compared using the Mann-Whitney *U* test for nonnormally distributed variables, and for normally distributed variables, compared using unpaired *t* test. To evaluate the relation between variables, Pearson's correlation coefficient in normally distribution, and Spearman's ranked correlation coefficient in nonnormally

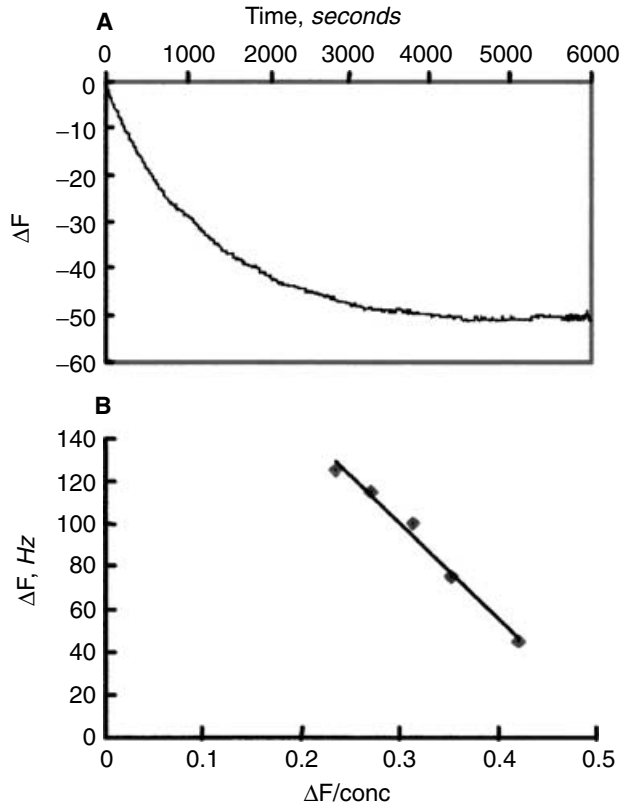

Fig. 3. Affinity of α_2-macroglobulin (α_2M) for β_2-microglobulin (β_2m) by quartz crystal microbalance (QCM). (*A*) Frequency changes (ΔF) of the QCM directory immobilized with β_2m responding to the addition of α_2M as described in the text (K_a 443.27 nmol/L, F_{max} 232.93 Hz, and ΔF_{max} 232.93 (Hz). (*B*) The Eadie-Hofstee plots ($y = -443.27x + 232.93$).

distribution were used. A P value less than 0.05 were considered as significant.

RESULTS

The affinity of β_2m for α_2M

The time course of the change in frequency of the QCM responding to 10 μL of 1 mg/mL α_2M was recorded (Fig. 3A). Association constants (K_a) and the maximum binding amount (ΔM_{max}) were calculated form Eadie-Hofstee plots (K_a 443.27 nmol/L, ΔM_{max} 232.93 Hz) (Fig. 3B).

β_2m exhibited a very strong affinity for α_2M in a dose-dependent manner (K_a 443.27 nmol/L, F_{max} 232.93 Hz). The frequency of the QCM responding to α_2M decreased over time, which indicated that α_2M had significant affinity for β_2m in a dose-dependent manner.

Serum levels of α_2M

Serum levels of α_2M were 151.5 ± 43.9 mg/dL in hemodialysis patients and 157.8 ± 50.3 mg/dL in prehemodialysis CRF patients. An increased level of serum α_2M greater than maximum reference value (200 mg/dL for males and 250 mg/dL for females) could be found only 14 out of 137 cases (10.2%). Serum α_2M values in the hemodialysis patients failed to correlate neither with the serum β_2m values or with the α_2M-β_2m complex (Fig. 4). Serum β_2m values showed also no correlation with the α_2M-β_2m complex (data not shown).

Serum levels of α_2M-β_2m complex

Serum levels of α_2M-β_2m complex could not be detected in serum from 15 normal persons, but could be detected in two out of 11 serums from the prehemodialysis CRF patients (18%), whose levels varied from 0 to 0.8 U/mL (0.09 ± 0.20 U/mL) (Fig. 5). In the hemodialysis patients, the complex could be detected in 95 out of 137 serums (69.3%), which varied from 0 to 1.0 U/mL (0.20 ± 0.24 U/mL). The complex level in the hemodialysis patients was significantly higher than that in the prehemodialysis CRF patients ($P = 0.013$).

Correlation between the serum α_2M-β_2m complex levels and clinical characteristics related to hemodialysis

Serum levels of α_2M-β_2m complex showed a correlation with the duration of hemodialysis ($\rho = 0.257$, $P = 0.043$) (Fig. 6) and an average value of the complex was significantly higher in the patients with long-term history of 12 years or more ($N = 57$) (0.28 ± 0.28 U/mL, 0 to 1.0 U/mL) than that in the patients with a hemodialysis history less than 12 years ($N = 80$) (0.15 ± 0.19 U/mL, 0 to 0.9 U/mL) ($P = 0.009$) (Fig. 7).

Furthermore, no correlation could be found between serum levels of α_2M-β_2m complex with the DRA scores in all hemodialysis patients, but serum complex levels were significantly higher in patients with high DRA score than that in patients with negative DRA scores (0.33 ± 0.32 U/mL vs. 0.16 ± 0.21 U/mL, $P = 0.018$) (Fig. 8). Even so, however, the time on hemodialysis was also significantly longer in the former than in the later, 218.7 months vs. 81.3 months ($P < 0.0001$).

Serum complex levels in patients who underwent an operation of carpel tunnel syndrome were 0.32 ± 0.32 U/mL, which was slightly, but not significantly, higher than 0.19 ± 0.22 U/mL in patients who had not undergone an operation ($P = 0.065$) (data not shown).

An effect of hemodiafiltration on serum levels of the α_2M-β_2m complex

Serum levels of the α_2M-β_2m complex in 11 patients who had undergone hemodiafiltration for more than 6 months with the high-performance membrane (hemodiafiltration group) was compared with 34 patients who had no history of hemodiafiltration with the high-performance membrane (nonhemodiafiltration

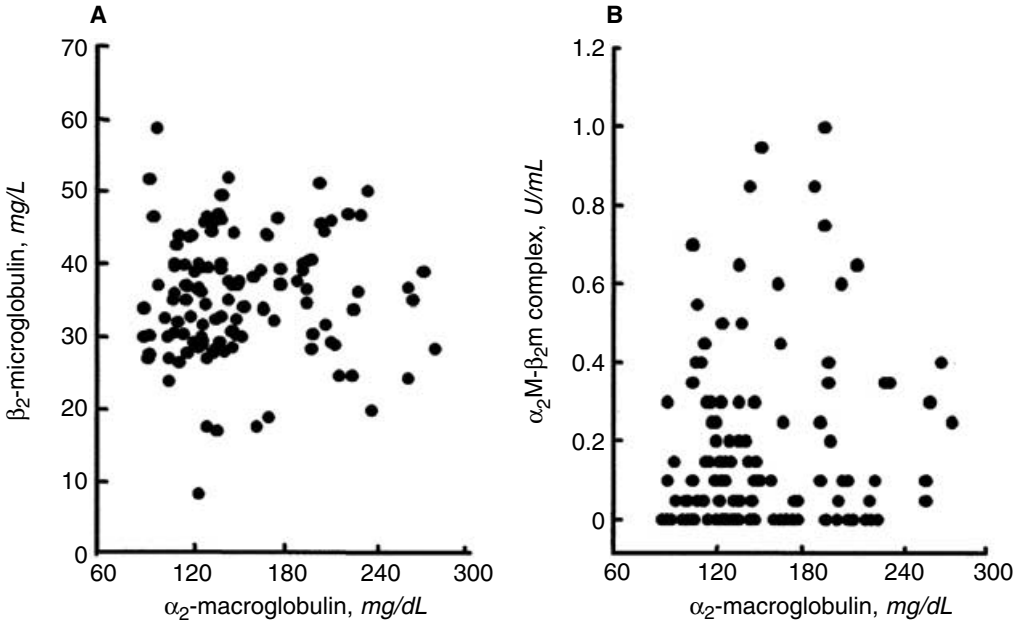

Fig. 4. Correlation between serum α_2-macroglobulin (α_2M) and β_2-microglobulin (β_2m) (*A*) and α_2M-β_2m complex (*B*) in hemodialysis patients. No significant correlation was found both of them ($N = 137$, $r = 0.024$, NS; $N = 137$, $\rho = 0.013$, NS).

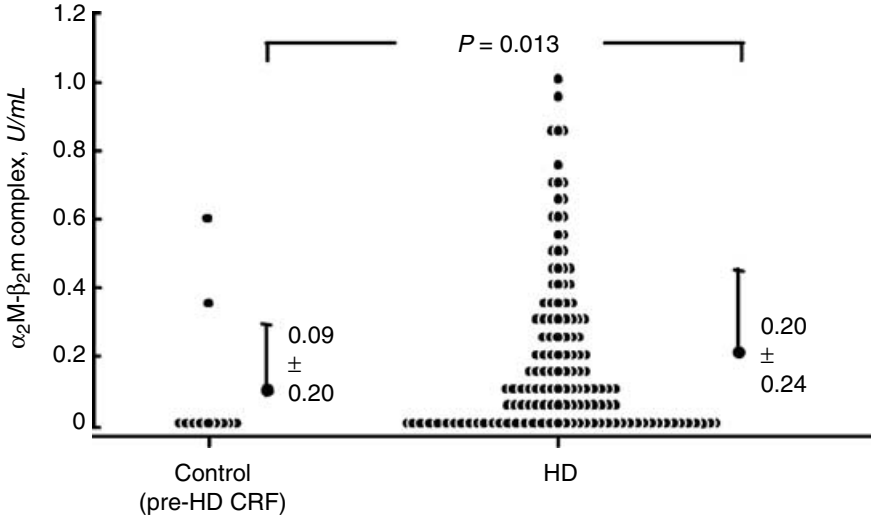

Fig. 5. Comparative study of serum levels of α_2-macroglobulin (α_2M)-β_2-microglobulin (β_2m) complex in the nonhemodialysis patients with chronic renal failure (CRF) (prehemodialysis CRF) ($N = 11$) and the hemodialysis (HD) patients ($N = 137$). In the hemodialysis patients, serum level of α_2M-β_2m complex was significantly higher than the prehemodialysis CRF patients (control subjects) (0.20 ± 0.24 U/mL vs. 0.09 ± 0.20 U/mL, $P = 0.013$).

group). In the nonhemodiafiltration group, the duration of hemodialysis was matched with the hemodiafiltration group. Duration of hemodialysis was 203.8 ± 51.6 months in the hemodiafiltration group and 205.1 ± 32.8 months in the nonhemodiafiltration group ($P = 0.920$). Age was 54.5 ± 6.6 years in the hemodiafiltration group and 54.4 ± 8.7 years in the nonhemodiafiltration group, respectively ($P = 0.955$). Serum values of β_2m were 32.8 ± 7.7 mg/L in the hemodiafiltration group and 35.4 ± 6.1 mg/L in the nonhemodiafiltration group ($P = 0.252$) and serum levels of α_2M were 156.0 ± 59.4 mg/dL in the hemodiafiltration group and 152.2 ± 36.4 mg/dL in the nonhemodiafiltration group ($P = 0.818$). Serum values of α_2M-β_2m complex were from 0 to 0.3 U/mL (0.05 ± 0.09 U/mL) in the hemodiafiltration group, which were significantly lower than not only those in the nonhemodiafiltration group (from 0 to 1.0 U/mL, 0.31 ± 0.29 U/mL) ($P = 0.002$) (Fig. 9), but also those in all hemodialysis patients included in this study (0.20 ± 0.24 U/mL) ($P = 0.009$). Taking into account this significant lower value of serum complex in the hemodiafiltration patients, a correlation between serum complex levels and DRA score was further studied in a patient subgroup excluding 11 hemodiafiltration patients, which

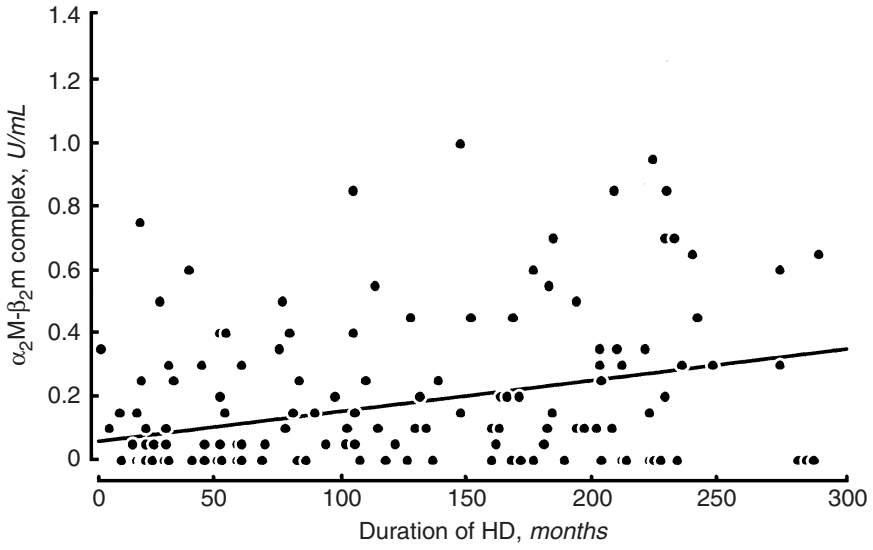

Fig. 6. Correlation between the duration of hemodialysis (HD) and serum α_2-macroglobulin (α_2M)-β_2-microglobulin (β_2m) complex level in 137 patients. Spearman's correlation $\rho = 0.257$, $P = 0.043$.

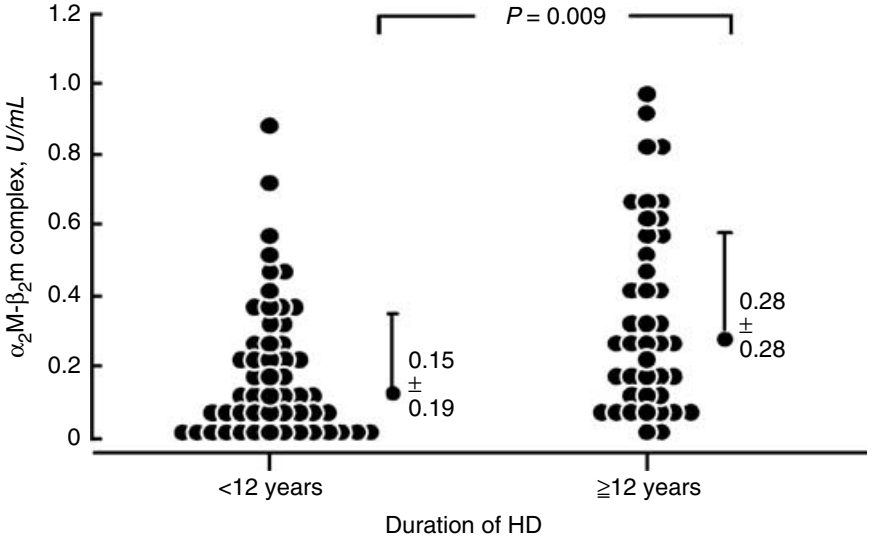

Fig. 7. Comparative study of serum levels of α_2-macroglobulin (α_2M)-β_2-microglobulin (β_2m) complex in the patient with long term history of hemodialysis (HD) with more than 12 years (≧12 years) ($N = 57$) and counter hemodialysis patients (<12 years) ($N = 87$). In the patient with long-term history of hemodialysi, serum α_2M-β_2m complex levels were higher than the counter patient group (0.28 ± 0.28 U/mL vs. 0.15 ± 0.19 U/mL) ($P = 0.009$).

showed a significant correlation as shown in Figure 10 ($\rho = 0.316$, $P = 0.0004$).

DISCUSSION

The presence of α_2M in amyloid tissue from patients with DRA was first demonstrated by Argilés et al in 1989 [3] and later corroborated by Campistol et al [4]. Thereafter, the complex formation of β_2m with α_2M was confirmed directly in an in vitro setting [9] and the α_2M hypothesis for the pathogenesis of DRA was proposed [10]. Thus far, however, no report concerning a circulating complex of α_2M-β_2m in hemodialysis patients has been published.

At present study, we could confirm that α_2M showed high affinity for β_2m in vitro by QCM analysis, a new method for binding assay [13, 14] and detected β_2m associated with α_2M (α_2M-β_2m complex) in serum from 95 out of 137 hemodialysis patients (69.3%). Circulating levels of α_2M were reported to be significantly increased and had a correlation with the serum levels of β_2m in hemodialysis patients [15, 16]. Interestingly, a significant correlation between serum levels of α_2M and β_2m was, however, limited to the patients with DRA in the study by Argiles et al [16]. By contrast to that study by Argilés et al, this study failed to show such correlation despite almost similar patient's background such as duration of hemodialysis. Two distinct differences could be pointed out between these two studies, one with serum levels of α_2M and another with proportion of patients with DRA. Our study involved apparently fewer patients with increase of serum α_2M as well as with DRA than the study of Argilés et al [16]. Not only was there no increase of serum α_2M but also no correlation between serum levels

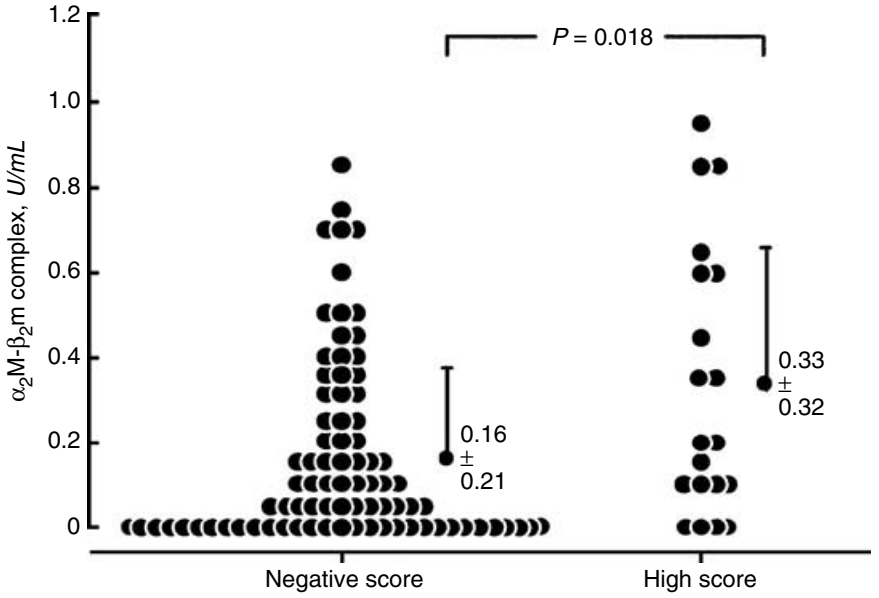

Fig. 8. Serum α_2-macroglobulin (α_2M)-β_2-microglobulin (β_2m) complex levels in patients with negative DRA score and patients with high dialysis-related amyloidosis (DRA) score. In the patient with high DRA score, α_2M-β_2m complex level was significantly higher than negative DRA score ($P = 0.018$).

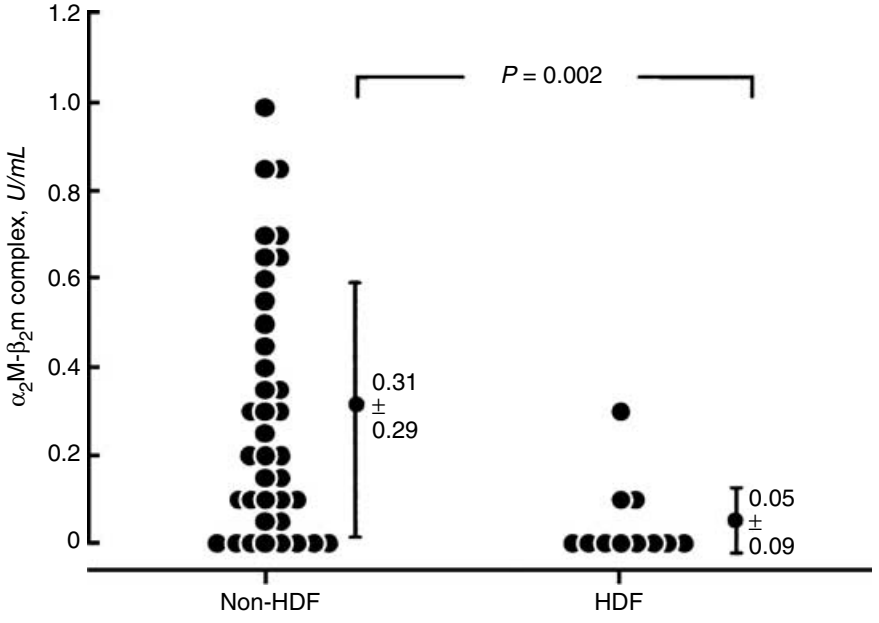

Fig. 9. Comparative study of serum levels of the α_2-macroglobulin (α_2M)-β_2-microglobulin (β_2m) complex in the hemodialysis (HD) group ($N = 34$) and treated the hemodiafiltration (HDF) group ($N = 11$). Serum level of the α_2M-β_2m complex in the hemodiafiltration group was lower than the hemodialysis group (0.05 ± 0.09 U/mL vs. 0.31 ± 0.29 U/mL, $P = 0.002$).

of α_2M and β_2m in patients free of DRA in their study similar to our study. On the other hand, Curatola et al [15] reported merely a significant increase of serum α_2M in hemodialysis patients. Recently, polymorphism of α_2M gene was reported [17], but to what extent it might be accountable for the difference in serum levels of α_2M on hemodialysis remains unclear.

It is generally acknowledged that β_2m is the key substance in DRA and that the development of DRA is strongly dependent on the length of time on hemodialysis, which is about 15 years on average in Japan [18]. In our study, a significant correlation was confirmed between the serum α_2M-β_2m complex levels and the duration of hemodialysis. However, we failed to find a correlation between the serum value of the α_2M-β_2m complex and the DRA score. In addition, patients involved in this study were extremely overbalanced by so many patients with a negative DRA score, the lower value of the serum α_2M-β_2m complex in the hemodiafiltration group (Fig. 9), which involved more patients with high DRA scores than in the hemodialysis group and may contribute to some extent to the lack of correlation with the DRA score. Actually, a significant correlation with DRA score and α_2M-β_2m complex could be confirmed in patient subgroup excluding hemodiafiltration patients ($P = 0.0004$) (Fig. 10).

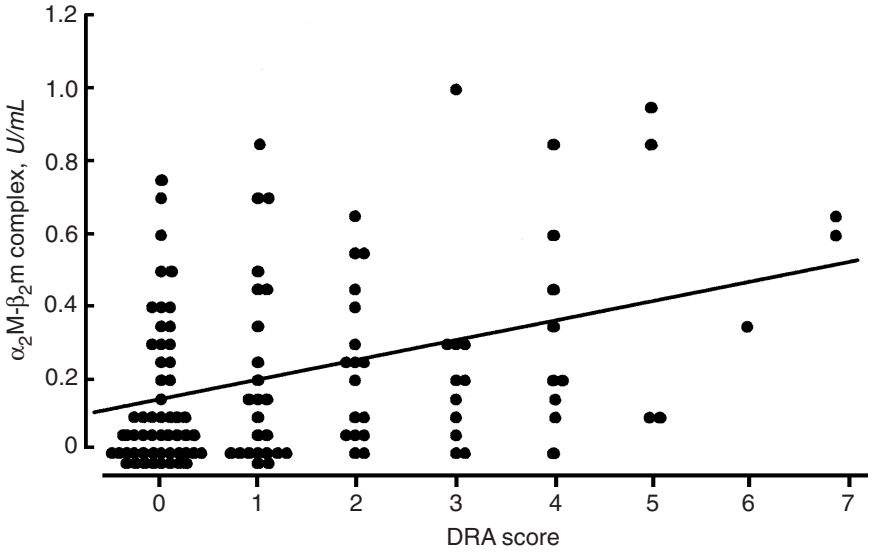

Fig. 10. Correlation between serum α_2-macroglobulin (α_2M)-β_2-microglobulin (β_2m) complex in hemodialysis patient subgroup excluding hemodiafiltration patients and dialysis-related amyloidosis (DRA) score. Spearman's correlation $\rho = 0.316$, $P = 0.0004$.

Although any direct inhibitory effect on protease by α_2M must still be substantiated in DRA amyloid tissue [19], Argilés et al [10] have given a clear indications that the α_2M-β_2m complex has great pathophysiologic significance in DRA and stressed the importance of an impaired catabolism of extravasated β_2m in amyloid tissue by protection from protease [10].

A kinetic study of β_2m using ^{125}I-β_2m suggested a progressive accumulation of β_2m in the extravascular space along with the time on hemodialysis [20, 21], while the serum β_2m concentrations tended to level off [22].

Because the bounding of the α_2M-β_2m complex was reported to be extremely tight, as indicated by an estimated kD of 10^{-9} mol/L [10], which was almost similar value in this study, a preformed complex is, therefore, assumed unlikely to be dissociated in the physiologic condition.

A molecular size of the complex is unlikely to be removed even by hemodiafiltration. Under such circumstance, circulating levels of the complex might be dependent on the extent by which the complex is generated.

Thus, it makes sense to assume that a lower value of serum complex in the hemodiafiltration group compared with the nonhemodiafiltration group may be due to lower β_2m value in those patients, albeit not significant, by high shuttling effect across the vascular wall of convection flow.

A recent report by Narita et al [23] suggested the possibility of a different process from the one involving the protection from protease, which is mediated via a low-density lipoprotein receptor-related protein (LRP). They have shown that the amyloid β peptide-α_2M complex is degraded by glioblastoma cells and fibroblast via LRP. Although it is not clear to what extent this process contributes to α_2M-β_2m catabolism, it is well-known that the involvement of activated macrophages would be ubiquitous in the late stages of DRA [24–26]. α_2M is also reported to bind to LRP on macrophages. Accordingly, an analogous possibility could be suggested in catabolism of α_2M-β_2m complex.

As shown in a statement by Glabe [27] concerning the up-to-date report by Iwata et al [28], the overall evidence from an investigation on the catabolic breakdown of amyloid proteins can be anticipated to provide new insights into the pathogenesis of the amyloidosis.

Of greater interest, the binding site of β_2m to α_2M was reported to be folded in the physiologic state [9]. The complex, therefore, may indicate the presence of an unfolded β_2m, which suggests a conformational change in this molecule, misfolding, in vivo [29].

Finally, as stated in a paragraph compared with the study by Argilés et al, this study involved many patients free of clinical evidences of DRA, which might enable us to undertake further study on prognostic value of α_2M-β_2m complex in progression of DRA.

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